Total Synthesis of Alvaradoins E and F, Uveoside, and 10-epi-Uveoside

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ABSTRACT: Concise total syntheses of the anthracenone C-glycosides alvaradoins E and F, uveoside, and 10-epi-uveoside (1–4) have been accomplished from chrysophanic acid 8 and bromosugar 9. Key steps in the syntheses include the DBU-induced coupling of 8 and 9 to produce β-C-glycoside 11, and a Pb(OAc)₄-mediated Kochi reaction to introduce the C-1′ oxygen atom of the natural products. Isothermal titration calorimetry and fluorescence binding studies reveal that compounds 1 and 2 have good affinity for the plasma protein HSA.

The anthracenone C-glycosides alvaradoin E (1, Figure 1) and alvaradoin F (2, Figure 1) were isolated from the leaves of the tropical tree Alvaradoa haitiensis in 2005 and 2007.¹ Both substances exhibited pronounced cytotoxicities toward human oral epidermoid carcinoma (KB) cell lines (EC₅₀(1) = 0.050 μM; EC₅₀(2) = 0.065 μM) among others; furthermore, alvaradoin E was demonstrated to induce apoptosis of cultured LNCaP cells. These results prompted the investigators to evaluate the in vivo activity of 1 and 2 in the P388 murine lymphocytic leukemia model. Alvaradoin E showed antileukemic activity (125% T/C) at a dose of 0.2 mg/kg per injection when administered intraperitonally. Uveoside (3, Figure 1) was isolated in 1998 from the chloroform extract of the roots of Picramnia antidesma by Hernandez-Medel and co-workers;² further work on the root bark of Picramnia antidesma by the same research group resulted in the isolation (in 2002) of 10-epi-uveoside (4, Figure 1), a substance also displaying elevated cytotoxicity toward KB cells.³ Given the heightened biological profile of this family of C-glycoside natural products, together with the fact that there is only a single previous total synthesis of a related anthrone C-glycoside,⁴ we decided to undertake a synthetic study of compounds 1–4.

One of the synthetic challenges anticipated en route to compounds 1–4 was the installation of the acid-labile anomeric C-1′ acetate and benzoate esters, a structural feature absent in the previously prepared anthrone glycoside cassialoin.⁵ We envisioned that a Hunsdiecker-type reaction⁶ on a carboxylic acid precursor would allow us to install the C-1′ oxygen atom in the form of a more stable acetal moiety, which could be transformed into the requisite C-1′ ester in the penultimate step of the synthesis. The realization of this plan is detailed in the present Letter.

Over the last two decades, there have been numerous advances in C-glycoside synthesis.⁷ Transition-metal catalyzed cross-coupling⁸ and metathesis⁹ reactions, [3,3]-sigmatropic

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Supporting Information
rrearrangements,17 and radical-olefin couplings18 have been used to prepare arene- and C(sp3)-linked C-glycosides. Since it is known that anthracenones undergo base-induced alkylation reactions at C-10,19 we decided to attempt a direct C-glycoside synthesis by combining anthracenones with C-1 bromosugars under basic conditions. Our initial studies employed anthralin (5, Scheme 1) as the nucleophile and bromoglucoside 6b20 as the carbohydrate electrophile for the substitution reaction. Treatment of an equimolar CH2Cl2 solution of 6b and 5 with 1 equiv of DBU resulted in a rapid disappearance of 6b, along with the formation of C-glycoside 7 on TLC. Upon isolation, it was found that compound 7 was formed in 42% yield as a 7:1 mixture of β and α stereoisomers at C-5'. Encouraged by this result, we prepared the bromogalactoside 9b21 by HBr/MeOH treatment of 1,6-di-O-acetyl-2,3,4-tribenzyloxygalactose 9a. Combination of 9b with 5 in the presence of one equivalent of DBU gave rise to a 65% yield (from 6a) of C-glycoside 10 as a single β-stereoisomer (>20:1 β:α) at C-5'. Analogously, treatment of a mixture of 9b and chrysophanol (8) with DBU provided a 51% yield (from 9a) of C-glycoside 11, again with the selective production (>20:1 β:α) of the C-5' β stereoisomer in excess; in addition, a 1:1 mixture of diastereomers was formed at C-10.14 TLC also showed the formation of oxidized aromatics (anthraquinones) as well as hydrolyzed carbohydrates (6 or 9, R2 = OH), accounting for the balance of the material in these reactions. All attempts to thwart their production by performing the reaction under vigorously anhydrous and anaerobic conditions led to no significant improvement in the yields of 7, 10, or 11 obtained.

In order to manipulate the hydroxymethyl group of the carbohydrate moiety to achieve installation of the C-1' oxygen atom, it was necessary to protect the C-1 and C-8 hydroxyl groups as sterically bulky esters that would be resistant to conditions for hydrolysis of the acetate ester of 11. While the bis-pivaloate derivative initially showed promise in this direction, late-stage removal of these esters under acidic conditions proved to be problematic (vide infra) and ultimately motivated a switch to the less bulky isobutyrate esters, which were installed by treatment of 11 with isobutyl chloride and triethylamine in CH2Cl2 (Scheme 2). Meth-
acetic acid, followed by treatment with silver benzoate and optical rotation data recorded for synthetic the natural compounds. Similarly, exposure of alvaradoins E and F were in accord with those reported for the natural compounds.29

Given that numerous C-aryl glycosides are known to form strong complexes with duplex nucleic acids,26 we undertook DNA binding studies with synthetic compounds 1–4 (see Supporting Information). Thermal denaturation studies21 with CT DNA were precluded by the fact that compounds 1–4 underwent decomposition in aqueous phosphate buffer solution (pH = 7.21) at elevated temperatures, as evidenced by UV spectroscopy. Therefore, utilizing fluorescence spectroscopy, we investigated the displacement of ethidium bromide (10 μM) from CT DNA (10 μM) by increasing concentrations of synthetic alvaradoins E and F, and found only a small effect on ethidium fluorescence emission intensity (at 590 nm) over the 0.01–100 μM concentration range of 1 and 2 (pH = 7.21, NaH2PO4/Na2HPO4 buffer); indeed, 85% of the initial fluorescence intensity of ethidium was measured at 50 μM of the ligands, and extrapolation of the data gave a C50 value of 240 μM. Similar results were obtained in ethidium displacement studies employing uveoside and 10-epi-uveoside (extrapolated C50 = 430 μM).22 Under otherwise identical experimental conditions, positive control netropsin produced a significant decrease in ethidium emission fluorescence intensity with a directly measurable C50 value of 10 ± 2.5 μM. These results indicate that, despite structural similarities to the C-aryl glycoside family of natural products, the anthracenone C-glycosides associate only weakly with duplex nucleic acids.

In light of these data, we explored the possibility that this family of C-glycosides may form complexes with proteins; indeed, synthetic C-glycosides have been previously shown to associate with plant-derived carbohydrate binding proteins.23 Titration of the abundantly available plasma protein human serum albumin24 (HSA; N form at pH 7) with alvaradoins E and F showed a marked quenching of the Trp 214 fluorescence emission at 388 nm (λex = 280 nm; Kapp = 2.79 ± 0.33 × 104 M−1; Ksv = 6.60 ± 1.42 × 104 M−1; see Supporting Information).25,26 A similar experiment performed with uveoside and 10-epi-uveoside revealed significantly less quenching of the Trp 214 fluorescence emission as compared to the alvaradoins and a notably weaker binding (Kapp = 6.11 ± 0.55 × 105 M−1; Ksv = 0.88 ± 0.36 × 105 M−1). Isothermal titration calorimetry27 (ITC) of HSA (57 μM) with alvaradoins E and F (500 μM solution, 25 °C, pH = 7.21, NaH2PO4/Na2HPO4 buffer) gave a binding constant Ksv of 3.01 ± 0.58 × 105 M−1, along with an enthalpy of binding, ΔH, of −11.7 ± 0.5 kcal/mol and an entropy of binding, ΔS, of −18.8 ± 2.2 cal/mol·K; similarly, ITC experiments with the uveosides showed only weak binding (Ksv < 1 × 104 M−1). HSA competition binding experiments employing warfarin (site I marker) or ibuprofen (site II marker) and the alvaradoins are currently underway to clarify which binding site (Sudlow site 1 or Sudlow site 2) on the protein is preferred by the anthracenone C-glycosides.28

In summary, we have developed short total syntheses of the anthracenone C-glycosides alvaradoins E and F, uveoside, and 10-epi-uveoside. Although these compounds display poor binding to duplex DNA, alvaradoins E and F show significant affinity for the plasma protein human serum albumin.

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Scheme 3. Preparation of Hemiacetal 16

Scheme 4. Completion of the Syntheses of 1–4

then afforded a 95% yield of alvaradoins E and F as a 1:1 mixture of diastereomers, which could be separated by careful and repeated column chromatography (SiO2, 98:2 → 94:6 CHCl3/MeOH). NMR (1H and 13C),29 mass spectroscopy (MS), and optical rotation data recorded for synthetic alvaradoins E and F were in accord with those reported for the natural compounds. Similarly, exposure of 16 to HBr in acetic acid, followed by treatment with silver benzoate and benzoic acid in CH2Cl2, gave rise to α-benzoate 18 in 76% yield. Hydrogenation then provided uveoside and 10-epi-uveoside in 90% yield as a 1:1 mixture of diastereomers, which could be separated by radial chromatography. Once again, NMR (1H and 13C), MS, and optical rotation data recorded for synthetic uveoside and 10-epi-uveoside were in accord with those reported for the natural compounds.29
**REFERENCES**


8. The C-5′ proton resonance in the 1H NMR spectrum of compound 10 at 3.69 ppm (doublet) displayed a coupling constant of 1.6 Hz and 9.4 Hz; the C-10 proton resonance of 10 at 3.69 ppm (doublet) displayed a coupling constant of 2.0 Hz. These data are indicative of the β-configuration at C-5′; see ref 16b and Procko, K. J.; Li, H.; Martin, S. F. Org. Lett. 2010, 12, 5632.

9. We attempted chromatographic separation (SiO2) of the C-10 diastereomers of compound 11 (as well as later compounds 17 and 18) but were unsuccessful. Since the diastereomers lead to different natural products, we proceeded with the mixtures until adequate chromatographic resolution could be achieved at the natural product stage, as indicated in the original isolation papers (ref 1).


11. (a) Sheldon, R. A.; Kochi, J. K. Org. React. 1972, 19, 279. (b) The C-1′ proton resonance in the 1H NMR spectrum of compound 14 (CDCl3) at 5.98 ppm (doublet) displayed a coupling constant of 2.4 Hz; C-1′ coupling constants of a similar magnitude were measured for compounds 17 and 18. These data are indicative of the α-configuration at C-1′. See: Karplus, M. J. Am. Chem. Soc. 1963, 85, 2870.


